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The Thaumarchaeota: an emerging view of their phylogeny and ecophysiology

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Thaumarchaeota range among the most abundant archaea on Earth. Initially classified as 'mesophilic Crenarchaeota', comparative genomics has recently revealed that they form a separate and deep-branching phylum within the Archaea. This novel phylum comprises in 16S rRNA gene trees not only all known archaeal ammonia oxidizers but also several clusters of environmental sequences representing microorganisms with unknown energy metabolism. Ecophysiological studies of ammonia-oxidizing Thaumarchaeota suggest adaptation to low ammonia concentrations and an autotrophic or possibly mixotrophic lifestyle. Extrapolating from the wide substrate range of copper-containing membrane-bound monooxygenases, to which the thaumarchaeal ammonia monooxygenases belong, the use of substrates other than ammonia for generating energy by some members of the Thaumarchaeota seems likely.

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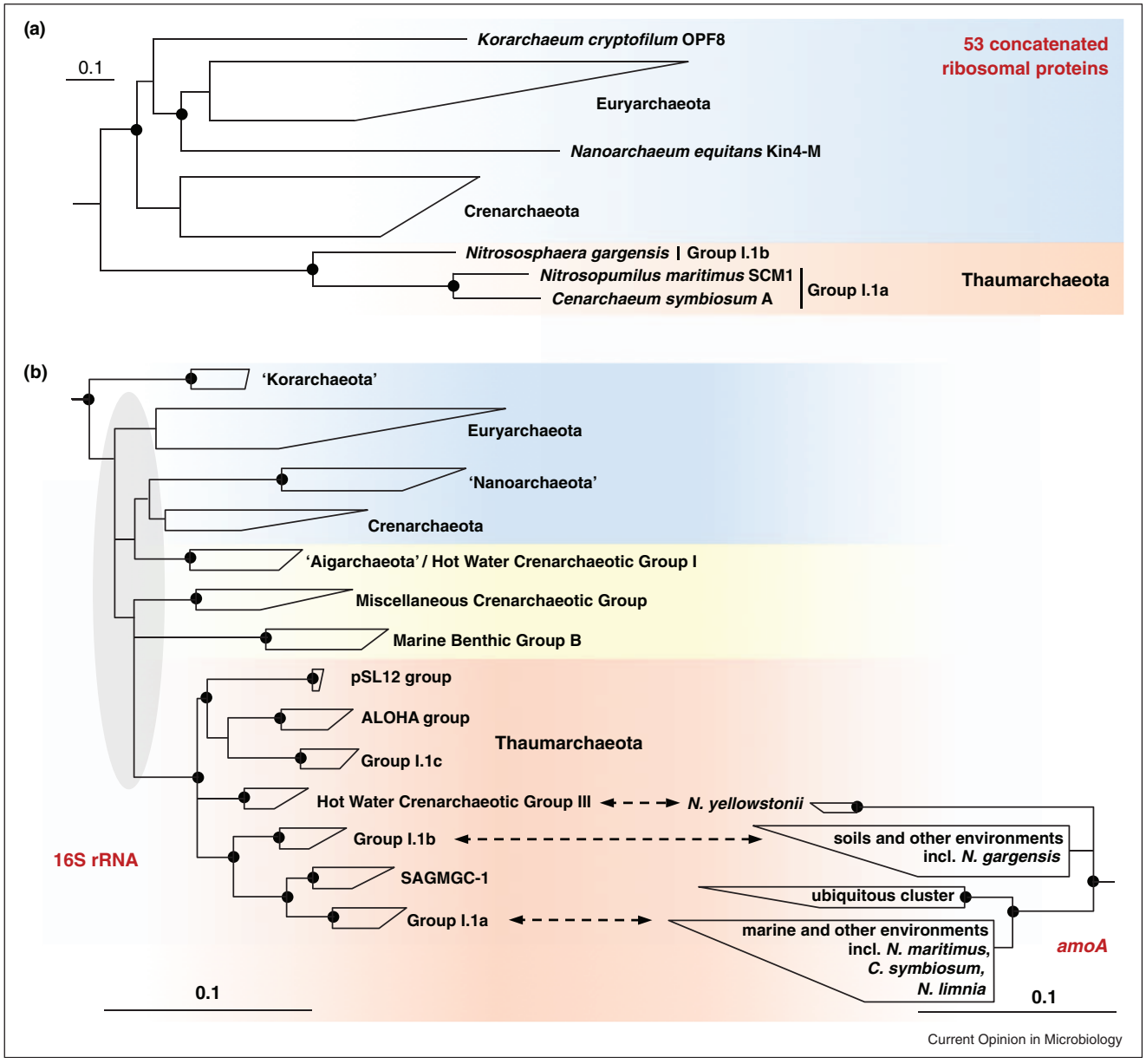
Introduction

Aerobic ammonia oxidation, the first and rate-limiting step in nitrification, is the only biological process converting reduced to oxidized inorganic nitrogen species on Earth [1]. For over 100 years, this process was thought to be mediated by autotrophic Beta-proteobacteria and Gamma-proteobacteria (AOB) [2] occasionally supported by heterotrophic nitrifiers in soil environments [3]. However, *in situ* measurements of nitrification in marine and terrestrial environments showed that ammonia oxidation often proceeds at substrate concentrations significantly below the growth threshold of cultured AOB (e.g. [4]) indicating the presence of unknown nitrifiers. The recent discovery of homologs of ammonia monooxygenase genes in archaea [5–7] and the cultivation of autotrophic ammonia-oxidizing archaea (AOA) [8–11] revealed that an additional group of microorganisms is able to catalyze this process. The widespread distribution of putative archaeal ammonia monooxygenase (*amo*) genes and their numerical dominance over their bacterial counterparts in most marine and terrestrial environments suggested that AOA play a major role in global nitrification [12–15], but our understanding of their evolutionary history and metabolic repertoire is still in its infancy.

From mesophilic Crenarchaeota to Thaumarchaeota

In 1992, Jed Fuhrman's team and Ed DeLong reported the discovery of a novel clade of archaeal 16S rRNA sequences from ocean surface waters, which formed a mesophilic sister group to the hyperthermohilic Crenarchaeota [16,17]. When it became apparent that this novel group contained AOA, these organisms were consequently also referred to as mesophilic Crenarchaeota. This perception was questioned by phylogenetic analysis of the first available genome sequence of a putative AOA, the sponge symbiont Candidatus *Cenarchaeum symbiosum*. When Brochier-Armanet and colleagues analyzed a concatenated data set of 53 ribosomal proteins common to Archaea and Eukarya, they made the surprising observation that *C. symbiosum* branched off before the separation of Crenarchaeota and Euryarchaeota. Based on this phylogenetic analysis, on gene presence/absence data, and on the diversity and wide distribution of AOA, they proposed that that these organisms belong to the phylum Thaumarchaeota [18••]. Recently, this analysis was extended to the ammonia-oxidizing Candidatus *Nitrosopumilus maritimus*, a marine group I.1a representative, and Candidatus *Nitrososphaera gargensis*, a soil group I.1b representative enriched from a hot spring. In this study, phylogenetic analysis of concatenated ribosomal proteins (Figure 1a) and several other marker genes as well as presence/absence patterns of information processing machineries in Archaea strongly supported the assignment of AOA to the deep-branching phylum Thaumarchaeota [19•]. Consistent with this finding, comparative genomics revealed that 6 conserved signature indels and >250 proteins are unique to the thaumarchaeota *C. symbiosum* and *N. pumilus* and are not found in Crenarchaeota [20]. Additional support for the phylum Thaumarchaeota stems from comparative analysis of fosmid

Figure 1



Phylogeny of ammonia-oxidizing Thaumarchaeota. **(a)** Schematic phylogeny of Archaea redrawn after a rooted maximum likelihood tree of 53 concatenated ribosomal proteins of Archaea (4853 deduced amino acid positions), with permission from Spang *et al*. [19•]. The scale bar represents 10% estimated sequence divergence. **(b)** Majority consensus trees based on the 16S rRNA gene (1067 nucleic acid positions conserved in >50% of all Archaea) and archaeal *amoA* gene (592 nucleic acid positions) as inferred by maximum likelihood, distance, and maximum parsimony methods. 'Nanoarchaeota' have been shown to represent a fast evolving lineage of the Euryarchaeota (reviewed by Brochier-Armanet *et al*. in this issue and **(a)**) and are misplaced in 16S rRNA-based trees as a sister group of the Crenarchaeota. The shadowed area highlights a region of the 16S rRNA tree with unstable branching order and low bootstrap support. The bars represent 10% Jukes-Cantor corrected sequence divergence. Dots (●) indicate in all trees bootstrap support above 80% as inferred by maximum likelihood. Abbreviations: *N. yellowstonii*, *Nitrosocaldus yellowstonii*; *N. gargensis*, *Nitrososphaera gargensis*; *N. maritimus*, *Nitrosopumilus maritimus*; *C. symbiosum*, *Cenarchaeum symbiosum*; *N. limnia*, *Nitrosoarchaeum limnia*, SAGMCG-1, South African Gold Mine Crenarchaeotic Group 1.

clones obtained from different deep-sea locations. Among 200 phylogenetic trees of protein families present in thaumarchaeotal fosmids from these sites, Thaumarchaeota sequences branched as separate cluster distinct from hyperthermophilic Crenarchaeota and Euryarchaeota in 162 phylogenetic trees [21]. Independent from genomic data, the presence of the lipid crenarchaeol in all analyzed AOA [9,22–24] is consistent with a separate

placement of these organisms in the archaeal tree as this lipid has so far not been found in any other bacterium or archaeon. Thus, it seems likely that this membrane lipid, which may now be more appropriately termed thaumarchaeol, is an invention of an early thaumarchaote and represents a signature lipid for this phylum.

Revisiting the phylogenetic placement of Thaumarchaeota in 16S rRNA-based trees also reveals a clear separation from Crenarchaeota and Euryarchaeota (Figure 1b). A number of environmentally retrieved clone groups consisting of the SAGMGC-1 group (subsurface mine), group I.1c (acidic soils), ALOHA group (open ocean), pSL12 group (hot spring), and the HWCGIII/*Nitrosocaldus* group (hot springs/hydrothermal vents) form a monophyletic cluster with known Thaumarchaota. Since this cluster is supported by all treeing methods and has a bootstrap value of 100% (Figure 1b), its representatives very likely all belong to the phylum Thaumarchaeota and at least some of them might be AOA. Supporting this hypothesis, a good correlation between copy numbers of archaeal *amoA* (coding for the α-subunit of ammonia monooxygenase) and 16S rRNA genes of the ALOHA group has been observed in the North Pacific [25]. It will be fascinating to see whether all Thaumarchaeota have the capability to perform ammonia oxidation or whether certain members use a different energy metabolism. Just recently two giant thaumarchaeota, Candidatus *Giganthauma karukerense* and Candidatus *Giganthauma insulaporcus*, were characterized by molecular methods but all attempts to amplify archaeal *amoA* genes failed [26•]. However, this could also be caused by primer bias as has been previously recognized for archaeal *amoA*-targeted surveys in deep ocean waters [27,28].

Currently, the MCG (Miscellaneous Crenarchaeotic Group), MBGB (Marine Benthic Group B), and HWCGI (Hot Water Crenarchaeotic Group I) clusters have no clear affiliation to any of the established archaeal phyla and show an unstable branching order when 16S rRNA-based trees inferred with different treeing methods are compared (Figure 1b). Little is known about these organisms but recently the first genome of a representative of the HWCGI cluster, that of Candidatus *Caldiarchaeum subterraneum*, was found to be distinct from other archaeal phyla including genes encoding a ubiquitin-like protein modifier system that was so far only found in eukaryotes. As a consequence, the lineage 'Aigarchaeota' was proposed [29••]. However, a comparative genome analysis by Brochier-Armanet and colleagues revealed some typical thaumarchaeal features in *C. subterraneum* and thus places it at the base of Thaumarchaeota in protein trees (for details see Brochier-Armanet *et al.*, this issue). With the availability of more genomes within this and related lineages, comparative genomics will show whether 'Aigarchaeota' represent a new archaeal phylum or will be classified as deep-branching members of the Crenarchaeota or Thaumarchaeota.

The phylogenetic structure of AOA can also be analyzed by the functional marker gene *amoA*, which is found in all ammonia-oxidizing microorganisms. The presence of AOA within Group I.1a and Group I.1b Thaumarchaeota as well as within the Thaumarchaeota-group HWCGIII/*Nitrosocaldus* is mirrored in the respective *amoA* phylogeny (Figure 1b). In addition, a fourth *amoA*-cluster with no established link to a thaumarchaeotal lineage in the 16S rRNA-based tree became apparent during the accumulation of environmental *amoA* sequences within the last few years. Since *amoA* sequences from a wide range of habitats (including various marine, terrestrial, and hot water environments) are affiliated with this lineage, we have named it the 'ubiquitous cluster'. It is tempting to speculate that this cluster represents so-far unrecognized AOA within the SAGMGC-1, group I.1c, ALOHA, or pSL12 cluster.

Emerging ecophysiology of Thaumarchaeota

Almost every study that investigates ammonia-oxidizing Thaumarchaeota uses the *amoA* gene to explore their diversity and abundance with the implicit assumption that all *amoA*-carrying archaea are oxidizing ammonia. However, of the >10,000 deposited archaeal *amoA* sequences, thus far only four have been directly linked to archaeal strains for which experimental evidence of ammonia oxidation exists [8–11]. Although phylogenetically closely related enzymes often perform the same function, it deserves consideration that the family of copper-containing membrane-bound monooxgenases (CuMMO), to which archaeal ammonia monooxygenases belong, has a wide substrate range. In addition to ammonia [ammonia monooxygenase (AMO) in β-Proteobacteria, γ-Proteobacteria, and Thaumarchaeota] [30], this includes methane [particulate methane monooxygenase (pMMO) in α-Proteobacteria, γ-Proteobacteria, Verucomicrobia, and Candidatus *Methylomirabilis oxyfera*] [31,32], and short-chained alkanes [particulate butane monooxygenase (pBMO) in the Gram-positive *Nocardioides* strain CF8] [33••]. In addition, non-specific substrate catabolism such as oxidation of chlorinated ethenes and aromatic hydrocarbons has been observed with some members of this enzyme family [34,35], clearly indicating substrate promiscuity. Therefore, it has been suggested that not necessarily the type of CuMMO but rather the downstream enzyme machinery defines the energy metabolism of a microorganism [36]. For example, the γ-Proteobacterium AOB *Nitrosococcus oceani* can oxidize methane but lacks all subsequent enzymes to gain energy by methane oxidation [37]. Likewise, co-oxidation of ammonia by methane oxidizing bacteria does not support their growth [38]. Furthermore, it is interesting to note that γ-proteobacterial AMOs are more closely related to γ-proteobacterial pMMOs than to β-proteobacterial AMOs and have a near equal substrate specificity for ammonia and methane [39]. Consequently, the mere presence of an *amoA*-like gene, transcript, or protein is insufficient to infer that the respective organism is oxidizing ammonia.

Currently, it is not clear whether AOA are strict autotrophs or also assimilate organic substrates. For *N. maritimus*, autotrophy has been shown [8] and for *N. gargensis* CO_2-fixation has been experimentally demonstrated [10]. Incorporation of labeled bicarbonate into lipids, proteins, and cells of marine thaumarchaeota [27,40,41] are consistent with autotrophy, which is enabled by a modified 3-hydroxypropionate/4-hydroxybutyrate (HP/HB) cycle for CO_2-fixation as found in known AOA genomes and in marine thaumarchaeal fosmids [7,42•,43,44]. However, analysis of the *C. symbiosum* and *N. maritimus* genomes as well as of thaumarchaeal fosmids from bathypelagic plankton also has revealed the presence of a TCA cycle (possibly incomplete) and of potential transporters for organic substances such as amino acids, oligopeptides, and glycerol [42•,43,45]. Thus, mixotrophic or even heterotrophic growth of marine Thaumarchaeota as supported by other isotope labeling studies and natural distribution of radiocarbon in archaeal membrane lipids [27,46,47] can to date not be excluded. Furthermore, it has been suggested that parts of the HP/HB cycle can serve to co-assimilate organic compounds including, for example, 3-hydroxypropionate, an intermediate in the metabolism of the ubiquitous marine osmoprotectant dimethylsulphoniopropionate [48•]. For soil environments, $^{13}CO_2$-stable isotope probing revealed ammonia oxidizing activity of members of group I.1a as well as I.1b Thaumarchaeota [49•,50,51•] indicating an autotrophic or mixotrophic lifestyle. Two of these studies found label incorporation into genes or transcripts of the 4-hydroxybutyryl-CoA-dehydratase [51•] or acetyl-CoA-propionyl-CoA-carboxylase [49•], respectively, with both enzymes being involved in the CO_2-fixing HP/HB cycle [48•]. However, growth of soil AOA with no concomitant incorporation of $^{13}CO_2$ has been also observed when nitrification was inhibited [52] indicating that at least some soil AOA can grow heterotrophically. For comparison, heterotrophic growth of Crenarchaeota that possess the HP/HB cycle is known for *Sulfolobus solfataricus* and *Metallosphaera sedula* with the latter being able to switch between an autotrophic and heterotrophic lifestyle [48•,53].

The question under which conditions AOA or AOB dominate ammonia oxidation is currently attracting a lot of attention. For ammonia oxidation by the group I.1a Thaumarchaeote *N. maritimus*, an extremely low substrate threshold (<10 nM total $NH_4^+ + NH_3$, representing the detection limit of the used method) and apparent K_m-value (133 nM) were determined with the latter being very similar to *in situ* nitrification measurements made in oligotrophic oceans [54••]. Adaptation to low ammonium concentrations has also been reported for the thermophilic group I.1b Thaumarchaeote *N. gargensis* [10], indicating a widespread distribution of oligotrophic ammonia oxidation within the Thaumarchaeota. In comparison, minimum total ammonium concentrations required for growth of cultured AOB are 100-fold higher (>1 μM near neutral pH) with K_m-values ranging from 46 to 1780 μM total ammonium [54••,55]. Thus, a dominating activity of AOA in the large water bodies of oligotrophic oceans is highly likely with AOB being restricted to organic-matter rich particles and coastal environments with higher nutrient loads [54••]. Measured apparent K_m-values for soils range from 2 to 42 μM total ammonium [54••,55] and may therefore be influenced by both AOA and AOB. In general, activity of soil AOA was seen when total ammonia concentrations were below 15 μg NH_4^+-N (g dw. soil)$^{-1}$ [30,49•] whereas AOB responded to high ammonia concentrations [>100 μg NH_4^+-N (g dw. soil)$^{-1}$] [49•,50,52,56]. In addition, the form of supplied nitrogen might also play a critical role: AOA activity was seen when N was supplied as mineralized organic N derived from composted manure or soil organic matter and AOB-dominated activity was seen with ammonia from inorganic fertilizer (reviewed in [30]).

Based on genome analyses of *N. maritimus* and *C. symbiosum* and due to the fact that AOA do not contain a homologue of the bacterial hydroxylamine oxidoreductase, a mechanism for ammonia oxidation distinctly different from that of AOB has been proposed. Here, ammonia is not oxidized via hydroxylamine (NH_2OH) as in AOB but rather via nitroxyl (HNO) to nitrite [42•], which possibly involves only 0.5 O_2 per NH_3 oxidized (proposed by Martin Klotz (Louisville) [30]). This hypothesized lower oxygen demand could explain why AOA are found not only in fully areated soils and oxic marine waters but also in suboxic marine waters, sediments, and oxygen-depleted hot springs [30,57]. In oxygen gradients of marine sediments and in the stratified water body of the Black Sea different AOA ecotypes were found to reside at different oxygen concentrations [58,59]. AOA can also be found over a wide range of pH, temperature, salinity, and phosphate concentrations with some AOA being adapted to sulfidic environments, which extends the potential range of AOA niche differentiation to a multitude of environmental factors (reviewed in [30,57]).

Conclusion and outlook

Until recently, methanogenic euryarchaeota were the only known archaea of global relevance for element cycling. This perception changed with the discovery of ammonia-oxidizing archaea, which belong to the newly recognized archaeal phylum Thaumarchaeota and contribute significantly to the global N-cycle and C-cycle. Their shear abundance in the ocean (up to 20% of all bacteria and archaea [60]) and extremely low substrate threshold for total ammonium provide compelling evidence for their role as dominant ammonia oxidizers in the open ocean, where they also contribute to primary production by their autotrophic (or possibly partly mixotrophic) lifestyle. The dominance of AOA over AOB in

many terrestrial environments cannot be so easily explained. Low K_m-values of unfertilized soils for ammonia oxidation [54••,55] might point to a contribution of certain AOA ecotypes to nitrification, especially under low ammonia availability. On the other hand, it is well possible that some soil thaumarchaeotes use other substrates than ammonia for energy generation and are heterotrophs or that they switch to ammonia oxidation only under certain environmental conditions. Future research is needed to investigate the ecophysiology of thaumarchaeota in greater detail. Further dissection of the ecological interplay of AOA groups among themselves and with AOB is urgently required and might reveal that AOA exhibit a similar type of niche partitioning as found for different nitrite oxidizers. Here, *Nitrobacter* spp. are known to dominate nutrient rich and oxygen saturated environments whereas *Nitrospira* spp. prefer low nutrient and microoxic sites [61], with different *Nitrospira* lineages adapted to different nitrite concentrations [62].

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